

**AMENDMENTS TO THE DRAWINGS:**

The attached sheet of drawings includes changes to Figure 4. The notation “n°” in “Figure n° 4” has been deleted from the drawing.

Attachments:        Replacement Sheet for Figure 4

**REMARKS**

Reconsideration of this application is respectfully respected.

**Status of Claims**

Claims 1-31 were presented for examination in this application. Claims 6-7 and 9-31 have been cancelled.

Claims 1-5 and 8 are presented for reconsideration.

**Priority**

Acknowledgment was made of Applicants' claim for foreign priority based on an application filed in France; however, the Examiner noted that Applicant has not filed a certified copy and the English translation of French application No. 03/00507 as required by 35 U.S.C. § 119(b). Office Action at 2. A verified English translation of French Application No. 03/00507 is filed herewith. Applicant respectfully requests that the translation be made of record.

**Amendments to the Specification**

Typographical and grammatical errors in the specification have been corrected. Specifically, the term "neuritic" has been changed to --neurite-- and the term "bonding" has been changed to --binding--. These amendments are consistent with the context of the specification in which these terms appear.

In addition, the term "intramedullary" has been changed to --intraspinal--, the expression "medullary compression" has been changed to --spinal cord compression--, and the expression "Medullary contusion model" has been changed to --Spinal cord contusion model--. These amendments are necessary to adapt the English translation of the application to the French priority application. Translation of the French

expression “*compression médullaire*” into “*medullary compression*” should have been “*spinal cord compression*.” The need for this change is apparent to the person of ordinary skill in the art based on the teachings in the specification.

Example 5 in the application describes the results of experiments using an animal model of trauma. The specification teaches that:

Two groups of rats were subjected to medullary [spinal cord] compression. Then, daily for 2 weeks, the animals received a subcutaneous injection containing either sesame oil alone (control group, n=20), or sesame oil containing molecule 43B (43B group, n=20; 12 mg/kg/day). The first injection was given 5 minutes after spinal cord compression. Locomotion of the animals, using BBB scores, was evaluated in a blind format on post-operation days 1, 4, 7, 14, 21, and 28.

Specification at page 18, lines 22-29.

A person skilled in the art would have recognized that the Applicant meant to use the term “spinal cord compression” rather than “medullary compression” because they used the BBB score to evaluate the efficiency of the treatment. This is evident from literature reports published before Applicant’s filing date.

For example, Parveen et al. reported that:

The purpose of this study was to assess the sensitivity of two commonly used behavioral scales, the 21-pt Basso, Beattie, and Bresnahan (BBB) Locomotor Rating Scale and the 5-pt Tarlov Scale, to determine locomotor recovery in a new rat model of lumbar spinal cord injury.

See 45<sup>th</sup> Annual Meeting, Orthopaedic Research Society, February 1-4, 1999, Anaheim, California, copy filed herewith.

Scheff et al. stated that:

The Basso, Beattie and Bresnahan (BBB) locomotor rating scale is widely used to test behavioral consequences of spinal cord injury (SCI) to the rat. Sensitivity of this rating

scale can differentiate hind limb locomotor skills over a wide range of injury severities.

See J. Neurotrauma, 2002 Oct; 19 (10): 1251-60, copy filed herewith.

Basso et al. stated that:

The BBB Locomotor Rating Scale offers investigators a more discriminating measure of behavioral outcome to evaluate treatments after spinal cord injury.

See J. Neurotrauma, 1995 Feb; 12(1): 1-21, copy filed herewith.

Similarly, Basso et al. stated that:

The Multicenter Animal Spinal Cord Injury Study (MASCIS) adopted a modified 21-point open field locomotor scale developed by Basso, Beattie, and Bresnahan (BBB) at Ohio State University (OSU) to measure motor recovery in spinal-injured rats.

See J. Neurotrauma, 1996 Jul; 13 (7): 343-59, copy attached.

As demonstrated in the accompanying abstracts, published before the priority date of the present application, the BBB score had been developed and was used to test behavioral consequences of spinal cord injury to the rat. Thus, it would have been clear to one of ordinary skill in the art that Example 5 relates to a model of spinal cord injury, and that the term "medullary" was an error.

Concerning the revision of "neuritic pain" to "neuralgia," neuritis is a lesion, while neuralgia corresponds to the pain induced by the lesion. Thus, Applicant submits that the proposed amendment is proper.

Concerning the replacement of "axon" by "neurite" in several occurrences of the description, mainly in the examples describing data obtained *in vitro*, Applicant believes that it would be obvious to one of ordinary skill in the art that the correct term is "neurite", since this term is usually used when referring to *in vitro* data obtained with

cultured cells. Indeed, as evidenced by attached pages from Webster's Online Dictionary, "neurite" corresponds to the French word "neurite" (the term used in the original French text), and is a more general term including both axons and dendrites, which is generally used when describing cells in culture (as is the case in the application), "because it can be difficult to tell axons from dendrites in that situation" (see Extended Definition: neurite).

### **Amendments to the Claims**

The claims have been amended as follows:

- Claim 1 is limited to a method using a composition with 3-methoxy-PREG to treat an acute or chronic spinal cord lesion;
- Claim 2 is directed to spinal cord compression; and
- Claims 6-7 and 9-31 have been cancelled.

Applicant submits that the limitation of all claims to only one compound, 3-methoxy-pregnenolone (3-methoxy-PREG), and the limitation to only one therapeutic use, spinal cord lesion, are supported by the Example in the application relating to spinal cord compression.

### **New Ground of Objection**

The drawing disclosure was objected to because the legend of "Figure n°4" should be corrected to "Figure 4". Office Action at 3. Figure 4 has been corrected. Accordingly, this objection may be withdrawn.

### **Claim Rejections - 35 USC § 112, first paragraph**

Claims 1-6, 8, and 14-31 were rejected for lack of an enabling disclosure. Office Action at 3. First of all, the Examiner contends that while claims 14 and 28 are enabled

for the compounds 3 $\beta$ -methoxy-pregna-5-ene-20-one (3-methoxy-PREG), the claims do not reasonably provide enablement for the other compounds: 3 $\beta$ -methoxy-pregna-5,14-diene-20-one; 3 $\beta$ -methoxy-PREG-16 $\alpha$ ,17 $\alpha$ -epoxy; and 3 $\beta$ -methoxy-PREG-16 $\alpha$ ,17 $\alpha$ -methylene. This ground for rejection is respectfully transverse.

The claims now read on a method of using a composition comprising 3-methoxy-PREG, which the Examiner indicated was supported by an enabling disclosure. Applicant reserves the right to claim the cancelled subject matter in a continuation application.

With regard to the treatment of Alzheimer's disease, the Examiner contends that there is a high degree of unpredictability in the state of the art regarding how to treat this disease. Office Action at page 7. According to the Examiner, one of ordinary skill in the art would be required to conduct an undue amount of experimentation to determine whether the claimed compounds in fact effectively treat Alzheimer's disease. Office Action at pages 8-9. Applicant courteously traverses this ground for rejection. Nevertheless, the claims have been amended by deleting Alzheimer's disease from the claims. Applicant reserves the right to claim the cancelled subject matter in a continuation application.

In addition, the Examiner contends that claims 1, 14, 19, 24, and 28 are "enabling for treating medullary trauma," but are not enabled for diseases, such as "a lesion of spinal cord" or "Alzheimer's disease." Office Action at 4. While Applicant courteously disagrees, this ground for rejection has been overcome by amending the specification and claims by replacing "medullary compression" with "spinal cord compression" and by deleting Alzheimer's disease from the claims.

In view of the foregoing amendments and comments, Applicant respectfully requests that the rejection under § 112, first paragraph, for lack of an enabling disclosure be reconsidered and withdrawn.

**Claim Rejections - 35 U.S.C. § 112 second paragraph**

Claim 2 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant previously amended claim 2 to recite that "the acute or chronic spinal cord lesion is medullary compression". The Examiner indicated that the amended claim is indefinite because the term "spinal cord lesion" is related to the spinal cord of the body; however, "medullary compression" is related to medulla of the body, which is also a part of the brain. Since the brain is not equivalent to the spinal cord of the body, the Examiner stated that it is unclear what Applicants intend to claim with respect to the disease treatment recited in claim 2. Office Action at 9-10.

This ground for rejection has been overcome by amending the claims and the specification by changing "medullary compression" to --spinal cord compression-- as previously discussed. Accordingly, the § 112, second paragraph, rejection may be withdrawn.

**Claim Rejections - 35 U.S.C. § 102**

Claims 19-31 were rejected under 35 U.S.C. § 102(a) as being anticipated by Baulieu et al. (EP 1310258). Office Action at 10. Applicant respectfully traverses this ground for rejection and requests reconsideration for the following reasons.

Baulieu is not prior art under 35 U.S.C. § 102(a). Baulieu was published on May 14, 2003, while Applicant's French priority application was filed on January 17, 2003. Thus, Applicant's effective filing date antedates the publication date of Baulieu. Applicant is filing herewith a verified English translation of French Application No. FR 03/00507 as required by 35 U.S.C. § 119(b). Accordingly, the rejection under § 102(a) may be withdrawn.

Applicant draws the Examiner's attention to the fact that EP 1 310258 is the priority application of PCT application WO 03/039554, which was filed in the English language on November 8, 2002, and published on May 15, 2003, but this PCT application does not seem to have entered U.S.

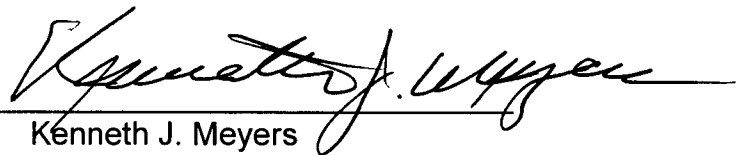
In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,  
GARRETT & DUNNER, L.L.P.

Dated: April 6, 2011

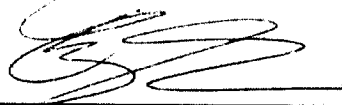
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**IN THE MATTER OF an Application  
for a French Patent in the name of  
MAPREG  
filed under No. 0300507  
published under No. 2 850 023**

**I, Cécile PUECH, c/o CABINET REGIMBEAU, 20 rue de Chazelles, F-75787 PARIS  
CEDEX 17, FRANCE do solemnly and sincerely declare that I am conversant with the  
English and French languages and that the following is, to the best of my knowledge and  
belief, a true and correct translation of the French Patent Application filed under No.  
0300507 on January 17, 2003 and published under No. 2 850 023 on July 23, 2004.**

**Date : March 2<sup>nd</sup>, 2011**



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**Cécile PUECH  
For and on behalf of  
CABINET REGIMBEAU**

The invention involves a novel use of neurosteroid derivatives, notably pregnenolone, to treat acute or chronic nervous system lesions, in particular certain neurodegenerative diseases, notably linked to the ability of  
5 the aforementioned neurosteroid derivatives to stabilize and/or increase the polymerization of neuronal microtubules.

A deterioration of neuronal cytoskeleton is observed in the majority of CNS lesions and neurodegenerative diseases. This deterioration can be the consequence but also the cause  
10 of damage to the affected cells. Indeed, microtubule depolymerization can be directly responsible for the dysfunction of certain neurons and can result in their death. Moreover, this deterioration affects the number and the length of the neuritic extensions of the remaining neuronal cells  
15 and, as a consequence, decreases their effectiveness. Treatment with NGF, which prevents dendritic atrophy, enables better functional recovery after a lesion of the cerebral cortex in the rat (Kolb et al., Neuroscience 1996). The degradation of the cytoskeleton observed after trauma to the  
20 CNS (Zhang et al., J Neuropathol Exp Neurol 2000) or an episode of ischemia, results from many factors, in particular the increase in glutamate and intracellular  $Ca^{++}$ , which involves microtubule depolymerization, and in the activation of proteases such as calpain which degrade MAP2. The use of a

calpain inhibitor (Schumacher et al., *J Neurochem* 2000) and the salting-out of glutamate (Springer et al., *J Neurochem* 1997) make it possible to decrease the consequences of spinal cord trauma in the animal by partially preserving the  
5 cytoskeleton.

Lesion repair recapitulates development. The existence of stem cells in certain regions of the central nervous system is well established today. Lesions stimulate the proliferation of these cells. However, these cells must migrate and  
10 differentiate. Differentiation implies, at a fundamental level, the development of the cytoskeleton.

MAP2 proteins represent one of the major components of the proteins associated with neuronal microtubules. They are present in all the extensions which constitute the dendritic  
15 arborization of a neuron, an arborization whose importance for the establishment of synaptic connections is known (Matus, *Microtubules* 1994; Sanchez et al., *Prog.Neurobiol* 2000). MAP2 proteins are absolutely necessary for the formation of dendrites as has been demonstrated in work by the authors who,  
20 by the suppression of MAP2 synthesis caused either neuritic growth to stop in neurons in culture (Caceres et al., *Neuron* 1992) or dendritic growth to stop in MAP2 knockout mice (Harada et al., *J.Cell.Biol.* 2002). The synthesis of MAP2 proteins is not in and of itself sufficient to induce this  
25 dendritic growth process. Certain steroids such as estradiol or progesterone can induce an increase in MAP2 synthesis (Reyna-Neyra et al., *Brain Res.* 2002) without inducing spectacular morphological changes. On the other hand, certain molecules bound to MAP2 have the extremely important and  
30 original property of reinforcing the activity of this protein, namely its role in the activation of the tubulin polymerization process (Murakami et al., *Proc Natl Acad Sci*

USA 2000) and the establishment of microtubular structures of greater stability.

It has been shown recently that, after cerebral ischemia, stem cells could differentiate into neurons and become  
5 integrated with the existing neuronal circuits (Nakatomi et al., *Cell* 2002). The stimulation of axon growth in these cells by molecules that improve tubulin polymerization could increase their functionality.

In spite of much research, at present no specific targets  
10 other than MAPs have been identified for pregnenolone.

MAP2 protein is found primarily in neurons. The molecules bound to MAP2 thus have as a target the cells of the nervous system. It is probable therefore that they do not have a notable action on other cellular types in which the  
15 concentration of MAP2 is very low.

Studies that demonstrate an effect by pregnenolone (PREG) *in vivo* are very few but they suggest a beneficial role for this steroid. It was shown that PREG decreased the astrocyte  
20 reaction following a cerebral lesion (Garcia-Estrada et al., *Int J Devl Neuroscience* 1999) and in the case of the increased astrocyte size observed during ageing (Legrand and Alonso, *Brain Res.* 1998). It also contributed to improved functional recovery after a spinal cord trauma (Guth et al., *Proc Natl Acad Sci USA* 1994). PREG protects cells arising from a  
25 hippocampal line (HT-22) against toxicity induced by glutamate and the protein beta amyloid (Gursoy et al., *Neurochem Res.* 2001).

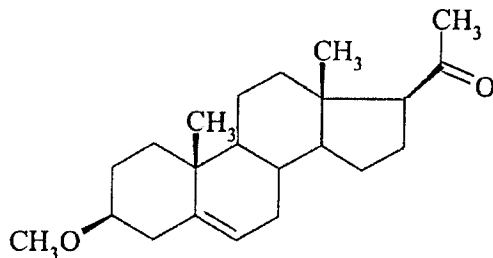
PREG is the precursor of all steroid hormones. Their  
30 synthesis implies the conversion of the PREG structure  $\Delta^5$ -3 $\beta$ -OH to  $\Delta^4$ -3-keto (implemented by an enzyme called 3 $\beta$ HSD). The Applicant blocked the  $\Delta^5$ -3 $\beta$ -OH structure to prevent its metabolism and also to prevent the formation of the ester

sulfate of PREG, a molecule that can be neurotoxic at high concentrations. Thus, the Applicant has revealed a compound, 3-methoxy-pregnenolone (3 $\beta$ -methoxy-pregna-5-ene-20-one, abbreviated as 3-methoxy-PREG), which possesses this property and which, moreover, is at least as active as PREG. The metabolic stability of this compound has been verified by mass spectrometry coupled with gas chromatography.

The Applicant considers as well that the invention is related to 3-methoxy-PREG, but also to all the molecules derived from pregnenolone that contain a 3-methoxy function or present a 3' function that can be converted into 3-methyl-ether. These molecules are incapable then of being converted into metabolites endowed with progestative (progesterone is a direct metabolite of PREG and, in addition to its hormonal activity, it is a PREG antagonist for the polymerization of microtubules), androgenic, estrogenic, and glucocorticoid activity. Also, they cannot be converted into ester sulfates which, like the sulfate of PREG, can have neurotoxic effects.

Within the scope of this invention, the Applicant has revealed the fact that 3-methoxy-PREG, or other molecules according to the invention, can play a major role in the polymerization and/or stabilization of microtubules, and presents quite remarkable activities for the treatment of pathologies related to the nervous system.

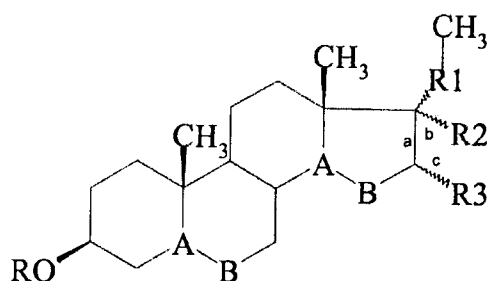
3-methoxy-PREG presents the following formula:



Thus, the invention relates to the use of 3-methoxy-PREG or a molecule derived from pregnenolone that contains a 3-methoxy function or that contains a 3' function that can be converted into 3-methyl-ether, for the preparation of a drug intended to treat an acute or chronic lesion or a degenerative disease of the nervous system, in particular those that accompany neurological, cognitive and neuropsychological deficits, or sensorial or sensory problems (notably neuralgias).

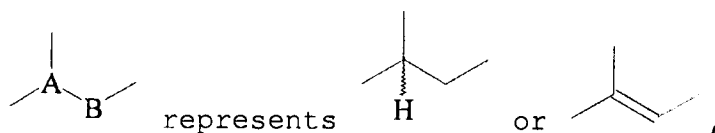
More generally, the invention relates to the use of a molecule of general formula I, for the preparation of a drug intended to treat an acute or chronic lesion or a degenerative disease of the nervous system, in particular those that accompany neurological, cognitive and neuropsychological deficits, or sensorial or sensory problems (notably neuralgias).

The molecules envisaged, derivatives of pregnenolone, in the context of the present invention, are of general formula I:



in which:

R = H or CH<sub>3</sub>,



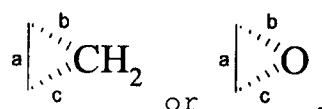
R1 = -CO-; -CH(OH)- or -CH(O-COCH<sub>3</sub>)-,

R2 = H or CHCl<sub>2</sub>,

R3 = H or CH<sub>3</sub>, or

R2 and R3 together form a ring:

5



In a preferred embodiment, R = CH<sub>3</sub>.

In a preferred embodiment, the aforementioned molecule is  
10 3-methoxy-PREG (3 $\beta$ -methoxy-pregna-5-ene-20-one).

In another embodiment, the aforementioned molecule is 3 $\beta$ -methoxy-pregna-5-ene-20-one-17- $\alpha$ -dichloromethyl.

In another embodiment, the aforementioned molecule is 3 $\beta$ -methoxy-5 $\alpha$ -pregnane-20-one.

15 In another embodiment, the aforementioned molecule is 3 $\beta$ -methoxy-5 $\alpha$ -pregnane-20 $\beta$ -ol.

In another embodiment, the aforementioned molecule is PREG-16 $\alpha$ -methyl.

In another embodiment, the aforementioned molecule is  
20 PREG-16 $\beta$ -methyl.

In another embodiment, the aforementioned molecule is 3 $\beta$ -hydroxy-pregna-5,14-diene-20-one.

In another embodiment, the aforementioned molecule is PREG-16 $\alpha$ ,17 $\alpha$ -epoxy.

25 In another embodiment, the aforementioned molecule is PREG-16 $\alpha$ ,17 $\alpha$ -methylene.

In another embodiment, the aforementioned molecule is Pregna-5-ene-3 $\beta$ ,20 $\beta$ -diol-20-acetate.

In another embodiment, the aforementioned molecule is 3 $\beta$ -  
30 hydroxy-5 $\alpha$ -pregnane-20-one-16 $\alpha$ -methyl.

In a particular embodiment, said molecule is a molecule as cited above in which the 3-hydroxy function has been changed to a 3-methoxy function.

5 In one embodiment, said disease is chosen from the group comprising Alzheimer's disease, age-induced memory loss (benign forgetfulness), memory loss induced by the taking of substances, a traumatic lesion, a cerebral lesion, a lesion of the spinal cord, in particular spinal cord compression,  
10 ischemia, pain, notably neuritic pain, nerve degeneration, and multiple sclerosis.

3-methoxy-PREG can also, within the scope of the present invention, be used to prepare a useful drug to treat other syndromes such as mental slowdown and loss of concentration,  
15 pain, including acute pain, post-operative pain, chronic pain, nociceptive pain, neuropathic pain, psychogenic pain syndromes, certain psychiatric states (notably depressive states), dissociative episodes including dissociative amnesia, dissociative fugue and dissociative identity disorder, and  
20 other neurodegenerative diseases such as Parkinson's disease, Huntington's disease, diseases related to prions, amyotrophic lateral sclerosis (ALS), and multiple sclerosis.

In a general way, 3-methoxy-PREG or the molecule derived according to the invention is used to treat any disease in  
25 which increased microtubule polymerization and/or stabilization is sought or is beneficial.

In a preferred embodiment, and notably to treat diseases related to a central nervous system disturbance, the aforementioned drug also comprises an excipient or a compound  
30 that makes it possible to formulate the aforementioned 3-methoxy-PREG such that it crosses the blood-brain barrier better. Such an excipient or compound can also make possible a

faster or more long-lasting crossing of the aforesaid blood-brain barrier.

Such an excipient or compound can be a peptide, such as the peptides described in application WO 00/32236, or 2-pyrrolidone.

The pharmaceutical compositions used in the invention can be administered by any route of administration including, but without being limited to, oral, intravenous, intramuscular, intraarterial, intraspinal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, and rectal.

A continuous or long-term treatment conducted directly via the cerebrospinal fluid using a pump implanted in the subarachnoid space in the brain or spinal cord can be envisaged. Such an implant could contain a concentrated solution of 3-methoxy-PREG (for example of cerebrospinal fluid or of cells constructed to overproduce and secrete a sufficient quantity of 3-methoxy-PREG).

Moreover, 3-methoxy-PREG can be administered with other compounds that contain biologically active agents (for example tensioactives, excipients, transporters, thinners and/or pharmaceutically acceptable vehicles). These compounds are well-known to those skilled in the art. Details on these chemicals can be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

In the pharmaceutical compositions provided by the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local, or rectal administration, the active ingredient (3-methoxy-PREG or a derived molecule) can be administered in unit dose formulations or in mixtures with traditional pharmaceutical media, applicable to animals or humans. Suitable unit dose administration formulations include oral route formulations

such as tablets, coated tablets, pills, capsules and soft gelatin capsules, oral powders, granules, solutions and suspensions, sublingual and buccal administration formulations, subcutaneous, intramuscular, intravenous, 5 intranasal, and intraocular administration formulations, and rectal administration formulations.

Pharmaceutical compositions can also contain preservatives, solubilizing agents, stabilizers, wetting agents, emulsifiers, sweeteners, dyes, flavoring, salts 10 intended to modify osmotic pressure, buffers, taste correctors, and antioxidants. They can also contain other therapeutically active substances.

Thus, pharmaceutical compositions according to the invention can also contain other neuroprotective agents, 15 notably compounds which increase neuronal regeneration. Such agents can be selected in particular from among the neuronal growth factors such as fibroblast growth factors (FGFs), acidic or basic, FGF-3, FGF-4, FGF-6, or keratinocyte growth factor (KGF). The addition of a neuroprotective agent can be 20 envisaged, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 or 4, TGF- $\beta$ 1, interleukins, or insulin-like growth factors (IGFs).

Any other types of neuroprotective antioxidant therapeutic agents can be used, notably glutamate inhibitors.

25 When a solid composition in tablet form is prepared, the principal active ingredient is mixed with a pharmaceutical vehicle such as gelatin, starch, lactose, stearic acid or magnesium stearate, talc, gum arabic or analogues. The tablets can be coated with saccharose or other suitable materials or 30 even be treated so as to have a prolonged or delayed activity and to release continuously a predetermined quantity of the active ingredient.

A capsule preparation is obtained by mixing the active ingredient with a thinner and pouring the mixture obtained into soft or hard capsules, with excipients such as vegetable oils, waxes, fats, semi-solid or liquid polyols, etc.

5       A preparation in syrup or elixir form can contain the active ingredient together with a sweetener, an antiseptic, as well as an agent giving taste and a suitable dye. Excipients can be used such as water, polyols, saccharose, invert sugar, glucose, etc.

10       Powders or water-dispersible granules can contain the active ingredient in a mixture with dispersing agents, wetting agents, and suspending agents, just as with taste correctors and sweeteners.

Suppositories, which are prepared with binders that melt  
15 at rectal temperatures, for example cocoa butter or semi-solid or liquid polyols such as polyethylene glycols, waxes, natural or hydrogenated oils, fats, etc., can be used for rectal administration.

For parenteral, intranasal, or intraocular  
20 administration, aqueous suspensions, isotonic saline solutions, or sterile, injectable solutions that contain pharmacologically compatible dispersing agents and/or wetting agents can be used. As an excipient, water, alcohols, polyols, glycerol, vegetable oils, etc., can be used.

25       The active ingredient can also be formulated in the form of microcapsules, possibly with one or more additive supports.

For the treatment of pain, topical application is the preferred route of administration. Here, the compositions according to the invention can be presented in the form of a  
30 gel, a paste, an ointment, a cream, a lotion, an aqueous or aqueous-alcohol liquid suspension, an oily solution, a dispersion of the lotion or serum type, an anhydrous or lipophilic gel, an emulsion with a liquid or semi-solid milk-

type consistency obtained by dispersing a fatty phase in an aqueous phase or vice versa, suspensions or emulsions of a soft or semi-solid cream- or gel-type consistency, or alternatively microemulsions, microcapsules, microparticles, or vesicular dispersions of the ionic and/or nonionic type. These compositions are prepared according to standard methods.

Moreover, a tensioactive can be included in the composition in order to enable deeper penetration by 3-methoxy-PREG.

Among the ingredients envisaged, the invention comprises agents enabling an increase in penetration selected, for example, from the group comprising mineral oil, ethanol, triacetin, glycerin and propylene glycol; cohesion agents are selected, for example, from the group comprising polyisobutylene, polyvinyl acetate, polyvinyl alcohol, and thickening agents.

Thus, in a first embodiment, the aforementioned drug is presented in an injectable form.

In another preferred embodiment, the aforementioned drug is presented in a form allowing oral administration.

Preferably, the aforementioned drug comprises an effective quantity of 3-methoxy-PREG, in particular ranging between 50 and 2500 mg by the parenteral route.

The aforementioned drug comprises preferentially an effective quantity of 3-methoxy-PREG or a molecule derived from pregnenolone that presents a 3-methoxy function, such that the quantity administered to the patient is comprised between 1 and 100 mg/kg.

An effective quantity of 3-methoxy-PREG is a quantity which allows the stabilization and/or polymerization of microtubules after administration to the host. Thus, the administration of an effective quantity of 3-methoxy-PREG results in the improvement or the elimination of the disease.

The quantity of 3-methoxy-PREG administered to the host will vary as a function of factors which include the height, age, weight, general health, sex, and diet of the host, the time of the administration, and the duration and characteristics of  
5 the disease associated with microtubule depolymerization/destabilization. For example, the dosage must reach, at the site of action, a concentration on the order of 0.5 to 100  $\mu$ M. The adjustment of dosages is well-known to those skilled in the art.

10 Thus, the invention relates to a therapeutic use of 3-methoxy-PREG. Thus, the invention relates to this compound as a drug.

A pharmaceutical composition comprising as an active ingredient 3-methoxy-PREG or a compound derived from  
15 pregnenolone having a 3-methoxy function, and a pharmaceutically acceptable excipient, is also an object of the invention.

The Applicant has revealed the activity of 3-methoxy-PREG which stabilizes and/or induces microtubule polymerization in  
20 a cell.

Thus, in a more general way, the invention relates to a method for increasing the stabilization and/or inducing the polymerization of the microtubules in a cell, comprising the step of exposing the aforementioned cell to the presence of 3-  
25 methoxy-PREG at a concentration from approximately 0.5 to 100  $\mu$ M, preferably 0.5 to 50  $\mu$ M. Microtubule polymerization can be indicated and marked by immunolabeling the MAP2 protein associated with these microtubules. Preferably, this method is implemented *in vitro*, but can be implemented *in vivo*, or *ex*  
30 *vivo* (on cells isolated from a patient, treated *in vitro* and reinjected) in certain cases.

The invention also relates to a method for increasing the growth of axons in a cell, comprising the step of exposing the

aforementioned cell to the presence of 3-methoxy-PREG at a concentration from approximately 0.5 to 50  $\mu$ M. This method is also implemented *in vitro* by preference, without excluding other modes of implementation if necessary.

5       The invention has also as an aim a method for reducing the depolymerization of microtubules and/or the retraction of axons in a cell, comprising the step of exposing the aforementioned cell to the presence of 3-methoxy-PREG at a concentration from approximately 0.5 to 50  $\mu$ M. This method is  
10 implemented *in vitro* also by preference, without excluding other modes of implementation if necessary.

      The invention also relates to a method for the treatment and/or the prevention of a disease induced or accompanied by the depolymerization of microtubules in a patient, comprising  
15 the step of the administration of an effective quantity of 3-methoxy-PREG to the aforementioned patient, or to a method for the treatment and/or the prevention of a neurodegenerative disease or lesion in a patient, comprising the step of the administration of an effective quantity of 3-methoxy-PREG to  
20 the aforementioned patient.

      Finally, a method to treat a patient after spinal cord compression or trauma, comprising the step of the administration of an effective quantity of 3-methoxy-PREG to the aforementioned patient, is also an object of the  
25 invention.

#### DESCRIPTION OF FIGURES

Figure 1: Kinetics of microtubule polymerization *in vitro*: effects of PREG (pregnenolone) and molecule 43B (3-methoxy-PREG). Purified MAP2 and tubulin are mixed in the  
30 presence of GTP at 4 °C in a spectrophotometer cuvette. Polymerization is induced by heating at 37 °C and is followed by the increase in optical density (OD) which indicates the

quantity of polymers formed. Lag time is decreased in the presence of PREG and molecule 43B, whereas the polymerization rate and the quantity of microtubules clearly increase compared to the control kinetics in the presence of solvent alone.

Figure 2: Effect of PREG and 3-methoxy-PREG (43B) on the average length of axons in PC12 cells. PC12 cells were cultured for 3 days in the presence of NGF (10 ng/ml) with or without (control) the addition of PREG or 43B molecules (30  $\mu$ M). Each molecule was tested in three culture wells. Measurements were taken for 200 cells per well using Scion Image software.

Figure 3: Dose-response relationship of molecule 43B on the average length of axons in PC12 cells. PC12 cells were cultured in the presence of NGF (10 ng/ml) and increasing concentrations of 3-methoxy-PREG (43B). Axon length was measured for 200 cells per well after 2, 5, and 8 days of culture.

Figure 4: Immunolabeling of microtubule-associated MAP2 in PC12 cells treated with PREG or 3-methoxy-PREG. PC12 cells were cultured in the presence of NGF (10 ng/ml) and PREG or 3-methoxy-PREG (20  $\mu$ M). They were fixed and exposed to anti-MAP2 antibodies that reveal microtubule-associated MAP2 exclusively.

Figure 5: Retraction of axons induced by nocodazole. After 7 days of culture in the presence of NGF (10 ng/ml), the cells were pretreated for one hour with PREG (30  $\mu$ M) or 43B (30  $\mu$ M), then exposed to nocodazole for 15 minutes (white columns: DMSO solvent alone; gray columns: nocodazole).

Figure 6: Effect of molecule 43B on locomotor recovery following spinal cord compression in rats. Animal locomotion was evaluated in a blind format during the 1-28 day post-operative period using the BBB score which evaluates the

degree of paralysis (higher values correspond to better recovery). Statistical significance: \* indicates  $p < 0.001$ ; \*\* indicates  $p < 0.0001$ .

Figure 7: Kinetics of the appearance of 3-methoxy-PREG (43B) in rat brain and spinal cord following subcutaneous injection of 43B (12 mg/kg) in a sesame oil solution.

The examples which follow are intended to illustrate the invention.

10

#### EXAMPLES

##### **Example 1: Synthesis of 3-methoxy-PREG**

10 g (52 mmol) of p-toluenesulfonyl chloride is added to a solution of 5g (15.8 mmol) of pregnenolone in 30 ml of pyridine. The mixture is stirred for 14 hours and then added to 100 ml of distilled water. After cooling the reaction medium to 0 °C, the mixture is filtered and the white solid obtained is dried under vacuum to yield 7.4 g (98 %) of pregnenolone tosylate.

20 The 7.4 g of pregnenolone tosylate is refluxed with methanol (50 ml) for 4 hours. After cooling and evaporation of the solvent, the crude reaction product is taken up in 100 ml of ethyl and washed 3 times with 100 ml of a 10 % sodium bicarbonate solution. After drying the organic phase over Na<sub>2</sub>SO<sub>4</sub>, it is evaporated dry under reduced pressure to yield 5.2 g (100 %) of 3-methoxy-PREG in the form of a white powder.

Pregnenolone can be obtained at low cost from commercial sources.

##### **Example 2: Test of 3-methoxy-PREG activity; comparison with PREG**

This *in vitro* test measures the effect of molecules on the polymerization of MAP2-induced microtubules. This

polymerization occurs when MAP2 proteins and tubulin are mixed at adequate concentrations in the presence of GTP. It is accompanied by an increase in optical density measured at 345 nm for 15 to 30 minutes with a UNICON spectrophotometer  
5 thermostated at 37 °C (Figure 1).

It is observed that molecule 43B, corresponding to 3-methoxy-PREG, activates microtubule polymerization as does pregnenolone (PREG). Other molecules, such as progesterone and pregnenolone sulfate, are PREG antagonists and do not  
10 stimulate polymerization (not shown).

### **Example 3: Cellular models**

#### *Effect of molecules on neurites' growth*

To test the effect of selected molecules on neurites' growth, we first used the PC12 line, which has long been  
15 employed in neurobiological research. In the presence of NGF (nerve growth factor), the cells of this line, which arise from a rat pheochromocytoma, form neuritic extensions containing MAP-associated microtubules. The growth of these  
20 elongations is stimulated by the addition of PREG. In the presence of PREG (30 µM), the increase in the average length of the axons after 4 days of culture reaches 60 %. The screening of other natural or synthetic steroids made it possible to select several molecules presenting greater  
25 effects than that of PREG (Figure 2). In particular, the addition of molecule 43B, which can be synthesized easily from PREG, caused a spectacular increase (reaching as high as 500 %) in the length of axons formed in the presence of NGF (Figure 3). This axon growth accompanies the stimulation by  
30 43-B of the association of MAP2 to the microtubules (Figure 4).

*Effect of steroids on the resistance of microtubules to nocodazole*

Nocodazole is a microtubule depolymerizing agent. Its addition to PC12 cell cultures, differentiated in the presence of NGF, causes axons to retract as a result of the depolymerization of their microtubules. Pretreatment of the cells by PREG or 43B makes the axons resistant to nocodazole due to an increase in the stability of their microtubules, a condition necessary for the formation of long axons (Figure 5).

**Example 4: Tests of toxicity**

*Cellular toxicity*

Cellular toxicity tests are carried out routinely on the PC12 cell line. The initial results show that PREG and 43B do not demonstrate toxicity at concentrations as high as their solubility limits (approximately 50  $\mu$ M).

*In vivo toxicity*

In rats, the daily injection for one month of 48 mg/kg of 43B (which is 4 times the active dose for spinal cord trauma) affected neither average weight nor behavior.

**Example 5: In vivo experiments - Spinal cord trauma**

*Spinal cord contusion model*

To determine the neuroprotective effects of the molecules tested, a spinal cord compression model is used. This model involves the total paralysis of the animals in the first few days following the operation. This period of paralysis is followed of a phase of approximately three weeks during which the animals partially recover their motor function. The study of this recovery using a simple and precise functional test based on observation of the animals (the BBB score) makes it

possible to study the speed and the degree of recovery of the animals, with and without treatment.

Two groups of rats were subjected to spinal cord compression. Then, daily for 2 weeks, the animals received a subcutaneous injection containing either sesame oil alone (control group, n=20), or sesame oil containing molecule 43B (43B group, n=20; 12 mg/kg/day). The first injection was given 5 minutes after spinal cord compression. Locomotion of the animals, using BBB scores, was evaluated in a blind format on post-operation days 1, 4, 7, 14, 21, and 28. Three animals in each group had to be excluded from the study. Statistical analysis of the results using the nonparametric Mann-Whitney test shows that the animals treated with 43B present results quite significantly higher than the control animals as of post-operation day 7 (Figure 6).

#### **Example 6: In vivo experiments - Cerebral ischemia**

Two models of cerebral ischemia in the rat were developed.

The first is a permanent or transient focal ischemia model of the middle cerebral artery using electrocoagulation or clamping (evaluation of neuroprotection by quantification of the volume of the lesioned area).

The second is a transient global cerebral ischemia model. This model is created in the rat by electrocoagulating and severing the vertebral arteries and then clamping the carotid arteries for a period of 15 minutes (evaluation of neuroprotection and cerebral plasticity increase by quantification of neuronal loss in the CA1 region of the hippocampus and by memory tests).

#### **Example 7: In vivo experiments - Alzheimer-type neurodegenerative disease model (transgenic mice)**

In order to evaluate the therapeutic potential of 43B to treat Alzheimer-type neurodegenerative diseases, a homozygous transgenic line of mice, such as described by Götz (EMBO J. 1995 Apr 3; 14(7):1304-13), can be used.

5        These mice express the longest human tau protein isoform. They present symptoms of neurological dysfunction expressed as muscular weakness and a reduction in motor coordination which correlate histologically with the appearance of abnormal axons and hyperphosphorylated tau proteins as is seen in Alzheimer's  
10       disease. This pathological phosphorylation decreases the affinity of tau for microtubules and favors its aggregation.

By treating these mice with molecules that increase microtubule stability, it is intended that the proportion of tau protein associated with the microtubules is increased and  
15       thus the appearance of symptoms is delayed.

#### **Example 8: In vivo experiments - Mnemonic performance**

##### *Mnemonic deficit induced by colchicine*

Colchicine, a substance which depolymerizes microtubules  
20       without blocking protein synthesis, is injected at very low doses that do not induce neuronal death in the rat hippocampus. These injections cause a learning deficit which results from lasting microtubule depolymerization. The objective is to test the effect of microtubule-stabilizing  
25       molecules on mnemonic deficits and histological lesions in the hippocampus induced by colchicine.

##### *Mnemonic deficit during ageing*

Studies on ageing are carried out on old rats presenting mnemonic deficits. The objective of this experiment is to  
30       mitigate these deficits by a chronic treatment with our molecules.

The two-step memory experiments are based on the spontaneous exploration of novelty and are adapted from the

experiments described by Dellsu et al. (1992, Brain Res., 588, 132-9) and Ladurelle et al. (2000, Brain Res., 858, 371-9). The technical instructions from these two publications concerning spatial memory tests using labyrinths are included  
5 in reference to the present application.

#### **Example 9: Pharmacokinetics**

The pharmacokinetics of the molecules tested *in vivo* are evaluated using gas chromatography/mass spectrometry (GC/MS)  
10 assays.

A study was conducted with PREG and molecule 43B. Its primary objective was to show that molecule 43B crossed the blood-brain barrier.

Rats were injected with either PREG or 43B diluted in  
15 sesame oil and assayed by GC/MS for the quantity of PREG or 43B in various organs at 1, 4, 8, and 24 hours after injection (12 mg/kg in 0.5 ml of sesame oil; subcutaneous injection).

The results presented in figure 7 show that molecule 43B penetrates rapidly into the spinal cord and the brain of the  
20 injected rats, and tends to accumulate there.

These results obtained *in vitro* and *in vivo* clearly demonstrate that molecule 43B (3-methoxy-PREG) gives spectacular results on the growth of axons in culture and on the spinal cord compression model.  
25

#### **Example 10: Other molecules according to the invention**

The indices of binding and activity are expressed as a percent of PREG.

Binding (affinity) is measured by the displacement of  
30 PREG-<sup>3</sup>H.

Activity is measured by the increase in optical density at 345 nm of a mixture of purified tubulin and MAP2, incubated at 37 °C in the presence of GTP.

Stimulation of neurites' sprouting is conducted on PC12 cells differentiated in the presence of NGF (10 ng/ml) and the steroid being tested (30  $\mu$ M) for 3 days. For each condition, the average length of the longest 200 axons in each cell is measured simultaneously for 3 cultures.

The results are represented by one, two or three crosses (+) according to whether stimulation is lower than, equal to, or higher than that produced by PREG.

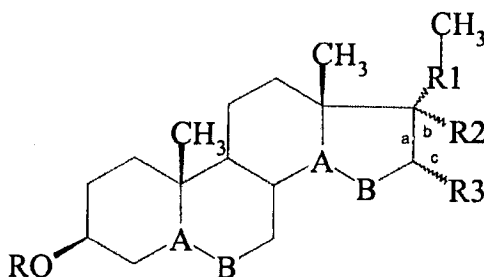
<b>Steroid</b>	<b>Affinity</b>	<b>Activity</b>	<b>Neurite sprouting</b>
<i>Pregnenolone (PREG)</i>	100	100	++
<i>3<math>\beta</math>-methoxy-pregna-5-ene-20-one</i>	100	100	+++
<i>3<math>\beta</math>-methoxy-pregna-5-ene-20-one-17<math>\alpha</math>-dichloromethyl</i>	53	113	+++
<i>3<math>\beta</math>-methoxy-5<math>\alpha</math>-pregnane-20-one</i>	87	10	+++
<i>3<math>\beta</math>-methoxy-5<math>\alpha</math>-pregnane-20<math>\beta</math>-ol</i>	65	65	++
<i>PREG-16<math>\alpha</math>-methyl</i>	80	70	++
<i>PREG-16<math>\beta</math>-methyl</i>	63	67	(++)
<i>3<math>\beta</math>-methoxy-pregna-5,14-diene-20-one</i>	102	50	+
<i>PREG-16<math>\alpha</math>,17<math>\alpha</math>-epoxy</i>	41	54	+
<i>PREG-16<math>\alpha</math>,17<math>\alpha</math>-methylene</i>	62	49	+
<i>Pregna-5-ene-3<math>\beta</math>,20<math>\beta</math>-diol-20-acetate</i>	60	108	++
<i>3<math>\beta</math>-hydroxy-5<math>\alpha</math>-pregnane-20-one-16<math>\alpha</math>-methyl</i>	57	53	(+)

10

These results show the effectiveness of other molecules derived from pregnenolone to stimulate the polymerization of microtubules induced by MAP2 and to stimulate neurite sprouting.

# Claims

1. The use of 3-methoxy-pregnenolone or a molecule derived from pregnenolone that contains a 3-methoxy function or that contains a 3' function that can be converted into 3-methyl-ether for the preparation of a drug to treat an acute lesion or a degenerative disease of the nervous system, with the aforementioned molecule presenting formula I:



in which:

R = H or CH<sub>3</sub>,

15 represents H or or ,

R1 = -CO-; -CH(OH)- or -CH(O-COCH<sub>3</sub>)-,

R2 = H or CHCl<sub>2</sub>,

R3 = H or CH<sub>3</sub>, or

R2 and R3 together form a ring:



2. The use according to claim 1, wherein the aforementioned disease is selected from the group comprising Alzheimer's disease, Parkinson's disease, age-induced memory loss, memory loss induced by the taking of substances, a

traumatic lesion, a cerebral lesion, a lesion of the spinal cord, in particular spinal cord compression, ischemia, pain, notably neuritic pain, nerve degeneration, and multiple sclerosis.

5

3. The use according to claim 1 or 2, wherein the aforementioned drug also comprises an excipient that makes it possible to formulate the aforementioned 3-methoxy-pregnenolone or derived molecule to cross the blood-brain barrier.

10

4. The use according to one of the claims 1 to 3, wherein the aforementioned drug is presented in an injectable form.

15

5. The use according to one of the claims 1 to 3, wherein the aforementioned drug is presented in a form allowing it to be taken orally.

20

6. The use according to one of the claims 1 to 5, wherein R is a CH<sub>3</sub> group.

7. The use according to one of the claims 1 to 6, wherein the aforementioned molecule is 3-methoxy-PREG.

25

8. The use according to one of the claims 1 to 7, wherein the aforementioned drug comprises a quantity of 3-methoxy-pregnenolone or of a derived molecule ranging between 50 and 2500 mg.

30

9. 3-methoxy-pregnenolone as a drug.

10. A pharmaceutical composition, comprising 3-methoxy-pregnenolone or a molecule derived from pregnenolone that

contains a 3-methoxy function or that contains a 3' function that can be converted into 3-methyl-ether of general formula I as an active ingredient, and a pharmaceutically acceptable excipient.

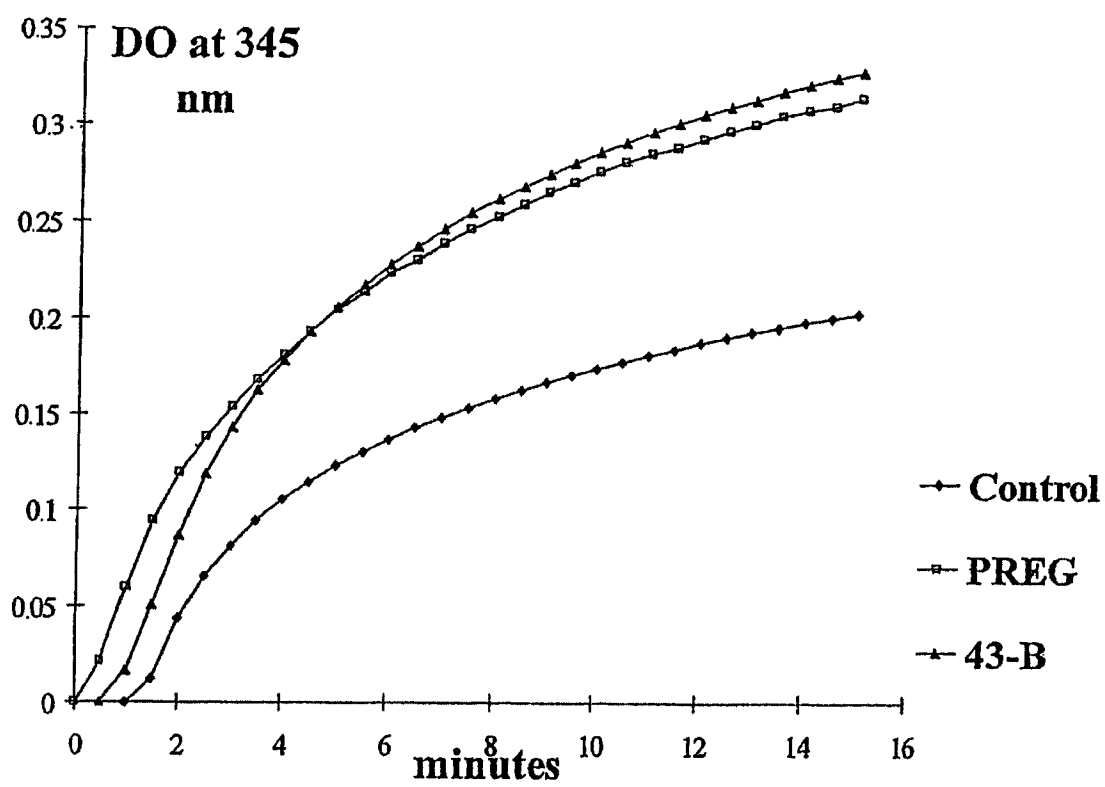
5

11. An *in vitro* method for increasing the stabilization and/or inducing the polymerization of the microtubules in a cell, comprising the step of exposing the aforementioned cell to the presence of 3-methoxy-pregnenolone at a concentration of approximately 0.5 to 50  $\mu\text{mol}$ .

10

12. An *in vitro* method for increasing neurites' sprouting in a cell, comprising the step of exposing the aforementioned cell to the presence of 3-methoxy-pregnenolone at a concentration of approximately 0.5 to 50  $\mu\text{mol}$ .

15

**Figure 1**

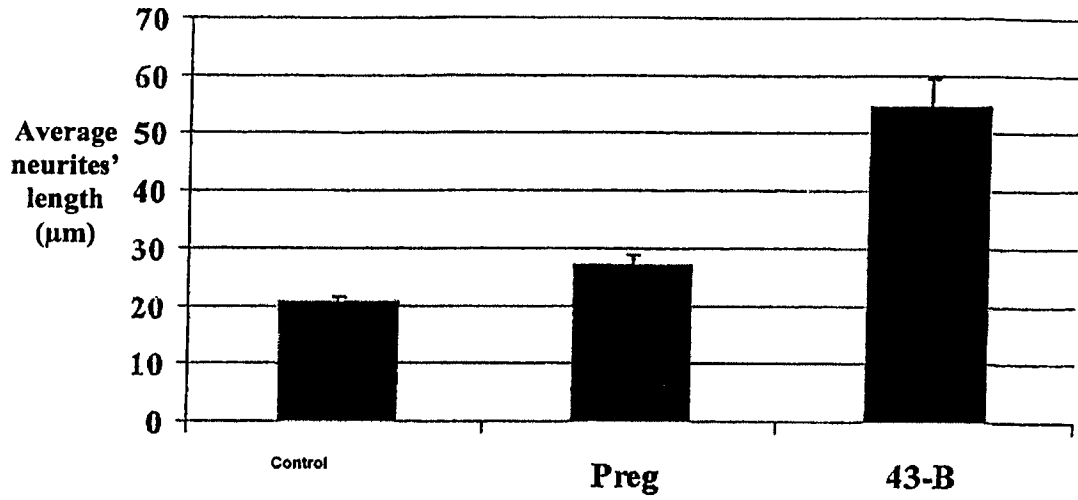


Figure 2

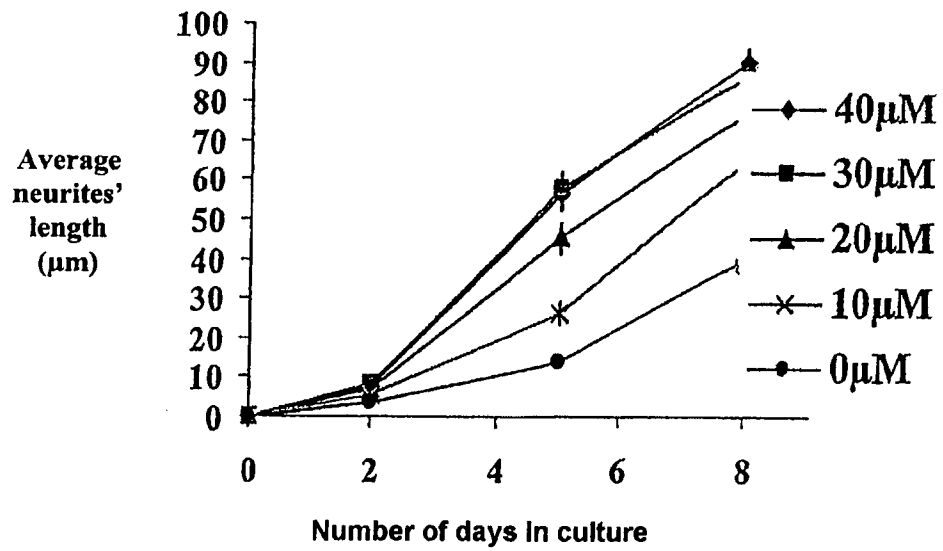
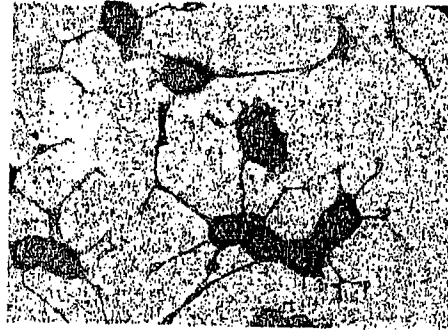


Figure 3

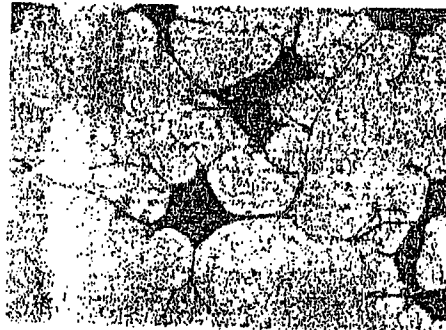
**NGF**



**NGF + Preg**



**NGF + 43B**



**Figure 4**

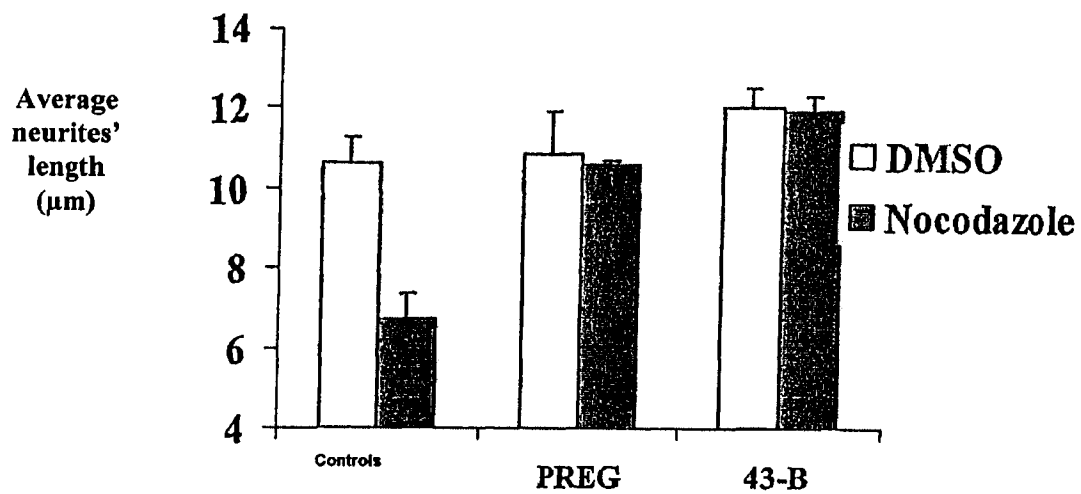


Figure 5

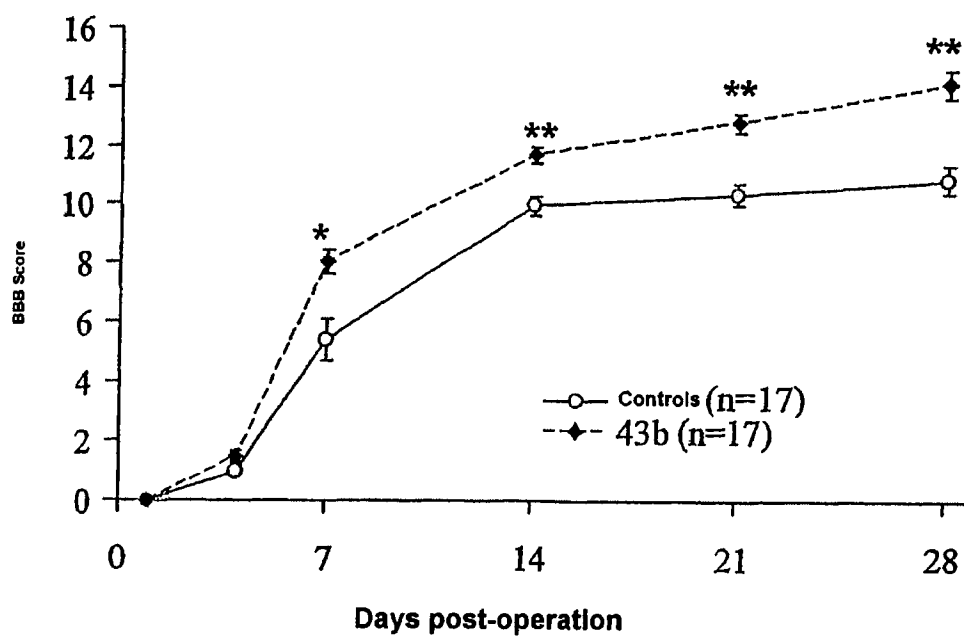
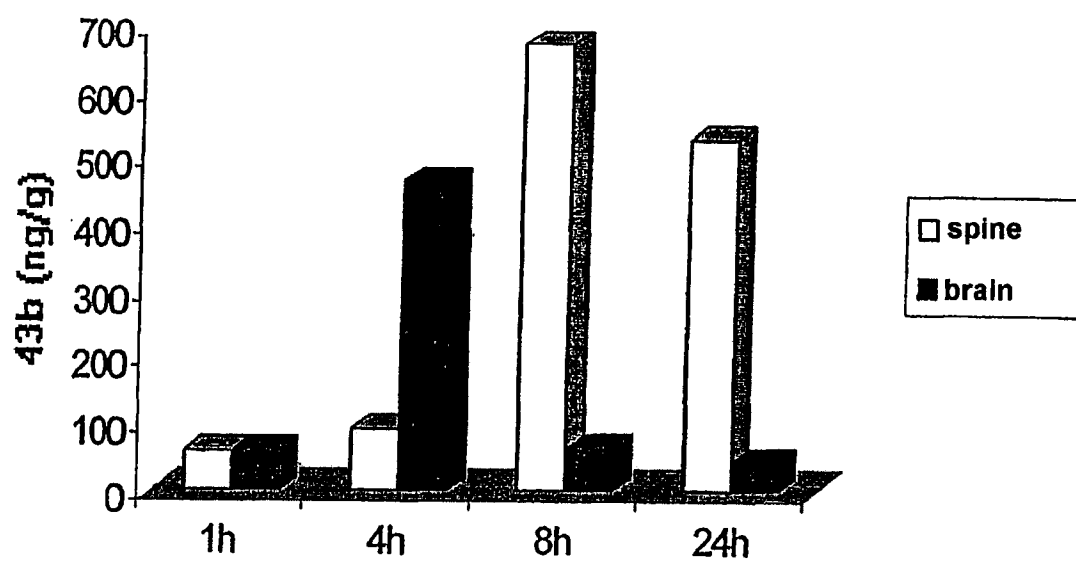


Figure 6

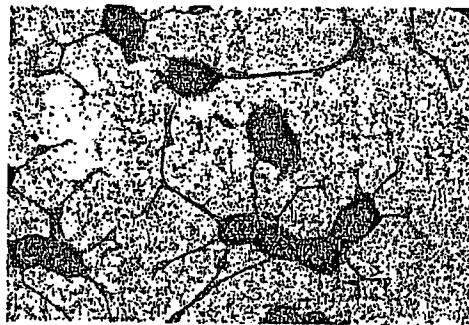
**Figure 7**

**Figure 4**

**NGF**



**NGF + Preg**



**NGF + 43B**



# EVALUATION OF LOCOMOTOR RATING SCALES FOLLOWING SPINAL CORD INJURY IN RATS

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## INTRODUCTION:

There are several rating scales used to assess recovery of locomotion following spinal cord injury, however, no single scale is used by all laboratories in this area of research. Therefore, comparisons of these studies can be difficult. The purpose of this study was to assess the sensitivity of two commonly used behavioral scales, the 21-pt Basso, Beattie, and Bresnahan (BBB) Locomotor Rating Scale and the 5-pt Tarlov Scale, to determine locomotor recovery in a new rat model of lumbar spinal cord injury.

## METHODS:

**Surgical Procedure:** Ten female Sprague-Dawley rats weighing 240-260gm underwent a concentric crush at the L2 vertebral level, resulting in a severe crush injury to the L2-L5 roots. 2-0 silk suture was passed ventrally underneath the spinal cord to create a loop. Tension was applied to both ends by adding weights for a determined amount of time. The injury groups were 100gm for 10 minutes (n=2), 100gm for 15 minutes (n=2), 125gm for 10 minutes (n=1) and 200gm for 20 minutes (n=5). The 100-125gm groups were considered low injury and the 200 gm group was considered high injury.

## Locomotor Rating Scales:

The *Basso, Beattie, and Bresnahan (BBB)* scale was developed at Ohio State University to measure recovery of locomotor function following spinal cord injury in rats. It is an ordinal 21-point scale, 0 being no observable hind limb movement and 21 being normal rat locomotion. The scale takes into consideration limb movement, trunk and abdomen position, paw placement and position, walking, and trunk instability.

The *Tarlov* scale is used to measure and record locomotor recovery following injury. It is often modified by different laboratories to better fit their injury models. Our Modified Tarlov scale ranged from a score of 0 to 5: 0, movement in the hip only; 1, movement in the hip and one other joint; 2, movement at all joints and non-weight bearing; 3, movement at all joints with partial weight bearing; 4, movement at all with full weight bearing yet abnormal; 5, normal locomotion.

**Behavioral Assessment:** Prior to injury, the rats were acclimated to the open field where behavioral observations were conducted. The protocol was similar to the BBB Open Field Training Procedures. Behavioral data was collected during four minute testing periods beginning post-operative day 3, and continuing once a week thereafter for 8 weeks. An investigator scored each hind limb individually according to the BBB and the Modified Tarlov scales.

## Statistical Analysis:

- The agreement between the BBB and Modified Tarlov scales was computed using the correlation coefficient at two different time points, post-operative day 3 and post-operative week 8, and between high and low injury groups.
- A ratio of behavior score given to total possible score was to adjust for the variable number of parameters between the two scales.
- A difference between the two ratios was considered lack of agreement between the two scales.
- Regression was applied to difference scores to assess the relationship with degree of injury.
- ANOVAs were calculated for each scale separately with one trial factor (repeated measure) and severity of injury as a grouping factor.
- Sufficient sample size was calculated for each scale using power analysis with the highest standard deviations and equivalent differences in scores: 4 points for the BBB and 1 point for the Modified Tarlov

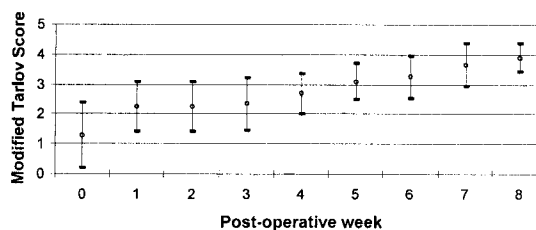
## RESULTS:

- The correlation coefficient for the low injury group was 0.95 at post-operative day 3 and 0.76 at post-operative week 8.
- The correlation coefficient for the high injury group was 0.88 at post-operative day 3 and 0.89 at post-operative week 8.
- Regression analyses on the difference scores did not show an association with the degree of injury.
- Comparing the two scales separately by ANOVA showed mean BBB scores were significantly different between post-operative day 3 and post-operative week 8 [  $F(1,6)=154, p<.001$ ]. In contrast, the mean Modified Tarlov scores did not differ at the two time points.
- There were no significant interactions between degree of injury and

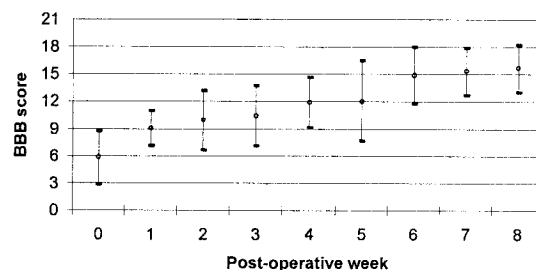
time.

- Using a power analysis and equalizing ratios, the determined sample size for a difference of four points on the BBB scale was n=6; while for a difference of one point on the Modified Tarlov scale, the sample size was n=13.

**Average Modified Tarlov Scores +/- Standard Deviation**



**Average BBB Scores +/- Standard Deviation**



## CONCLUSIONS:

The BBB and Modified Tarlov scores were highly correlated at early and late time points in both injury groups, yielding equivalent locomotor recovery ratios in all cases as determined by correlation coefficients. The difference in ratios, which reflect the lack of agreement between the two scales, were not significantly related to the degree of injury or the duration of recovery by regression analyses.

Although the two scales were highly correlated, the Modified Tarlov was not able to distinguish between behavioral scores at post-operative day 3 and post-operative week 8. However, the BBB scale, because of its greater detail, was able to discriminate significant differences between the two time points by ANOVA. This greater sensitivity of the BBB scale is reflected by a fifty percent decrease in the sample size necessary to demonstrate differences in behavioral scores.

The results of this study suggest that the BBB scale can serve as an excellent alternative to the more established Tarlov Scale, because of the high correlation of locomotor recovery data and the BBB's greater sensitivity for differences of lower magnitude in groups of smaller sample size. The BBB scale is easy to learn and we have chosen to use only the BBB scale at UCLA to assess locomotor recovery in our rat model of lumbar spinal cord injury.

## REFERENCES:

- Basso DM, Beattie MS, Bresnahan JC. A Sensitive and Reliable Locomotor Rating Scale for Open Field Testing in Rats. *J of Neurotrauma* 1995; 12(1):1-21.
- Tarlov IM, Klinger H. Spinal Cord Compression Studies. II. Time Limits for Recovery after Acute Compression in Dogs. *Arch Neurol Psychiat* 1954; 71, 271-90.

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## PubMed

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Display Settings: Abstract

J Neurotrauma. 2002 Oct;19(10):1251-60.

### **A statistical method for analyzing rating scale data: the BBB locomotor score.**

Scheff SW, Saucier DA, Cain ME.

Sanders-Brown Center on Aging, University of Kentucky, Lexington 40536, USA. sscheff@uky.edu

#### **Abstract**

The Basso, Beattie and Bresnahan (BBB) locomotor rating scale is widely used to test behavioral consequences of spinal cord injury (SCI) to the rat. Sensitivity of this rating scale can differentiate hind limb locomotor skills over a wide range of injury severities. While the 21-point BBB scale is ordinal in nature, the present discussion recommends the use of parametric statistics to evaluate the locomotor results. Specifically, it defines appropriate statistical analysis of these data in order to facilitate interpretation of results between laboratories and to provide a common methodology for the correct interpretation of SCI behavioral data.

PMID: 12427332 [PubMed - indexed for MEDLINE]

MeSH Terms

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## PubMed

Search: Basso DM[Author] AND 1995[Publication Date]

U.S. National Library of Medicine  
National Institutes of Health

Display Settings: Abstract

We found 1 article in 1995 by Basso DM:

J Neurotrauma. 1995 Feb;12(1):1-21.

### **A sensitive and reliable locomotor rating scale for open field testing in rats.**

Basso DM, Beattie MS, Bresnahan JC.

Department of Cell Biology, Neurobiology and Anatomy, Ohio State University, Columbus, USA.

#### **Abstract**

Behavioral assessment after spinal cord contusion has long focused on open field locomotion using modifications of a rating scale developed by Tarlov and Klinger (1954). However, on-going modifications by several groups have made interlaboratory comparison of locomotor outcome measures difficult. The purpose of the present study was to develop an efficient, expanded, and unambiguous locomotor rating scale to standardize locomotor outcome measures across laboratories. Adult rats (n = 85) were contused at T7-9 cord level with an electromagnetic or weight drop device. Locomotor behavior was evaluated before injury, on the first or second postoperative day, and then for up to 10 weeks. Scoring categories and attributes were identified, operationally defined, and ranked based on the observed sequence of locomotor recovery patterns. These categories formed the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale. The data indicate that the BBB scale is a valid and predictive measure of locomotor recovery able to distinguish behavioral outcomes due to different injuries and to predict anatomical alterations at the lesion center. Interrater reliability tests indicate that examiners with widely varying behavioral testing experience can apply the scale consistently and obtain similar scores. The BBB Locomotor Rating Scale offers investigators a more discriminating measure of behavioral outcome to evaluate treatments after spinal cord injury.

PMID: 7783230 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Grant Support

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## PubMed

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National Institutes of Health

Display Settings: Abstract

J Neurotrauma. 1996 Jul;13(7):343-59.

### **MASCIS evaluation of open field locomotor scores: effects of experience and teamwork on reliability. Multicenter Animal Spinal Cord Injury Study.**

Basso DM, Beattie MS, Bresnahan JC, Anderson DK, Faden AI, Gruner JA, Holford TR, Hsu CY, Noble LJ, Nockels R, Perot PL, Salzman SK, Young W.

Ohio State University, Columbus, Ohio 43210, USA.

#### **Abstract**

The Multicenter Animal Spinal Cord Injury Study (MASCIS) adopted a modified 21-point open field locomotor scale developed by Basso, Beattie, and Bresnahan (BBB) at Ohio State University (OSU) to measure motor recovery in spinal-injured rats. BBB scores categorize combinations of rat hindlimb movements, trunk position and stability, stepping, coordination, paw placement, toe clearance, and tail position, representing sequential recovery stages that rats attain after spinal cord injury. A total of 22 observers from 8 participating centers assessed 18 hindlimbs of 9 rats at 2-6 weeks after graded spinal cord injury. The observers were segregated into 10 teams. The teams were grouped into 3 cohorts (A, B, and C), consisting of one experienced team from OSU and two non-OSU teams. The cohorts evaluated the rats in three concurrent and sequential sessions. After viewing a rat for 4 min, individual observers first assigned scores without discussion. Members of each team then discussed and assigned a team score. Experience (OSU vs. non-OSU) and teamwork (individual vs. team) had no significant effect on mean scores although the mean scores of one cohort differed significantly from the others ( $p = 0.0002$ , ANOVA). However, experience and teamwork significantly influenced reliability of scoring. OSU team scores had a mean standard deviation or discordance of 0.59 points, significantly less than 1.31 points for non-OSU team scores ( $p = 0.003$ , ANOVA) and 1.30 points for non-OSU individual scores ( $p = 0.001$ , ANOVA). Discordances were greater at the upper and lower ends of the scale, exceeding 2.0 in the lower ( $< 5$ ) and upper ( $> 15$ ) ends of the scale but were  $< 1.0$  for scores between 4 and 16. Comparisons of non-OSU and OSU team scores indicated a high reliability coefficient of 0.892 and a correlation index ( $r^2$ ) of 0.894. These results indicate that inexperienced observers can learn quickly to assign consistent BBB scores that approach those given by experienced teams, that the scores are most consistent between 4 and 16, and that experience improves consistency of team scores.

PMID: 8863191 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms

LinkOut - more resources

## with Multilingual Thesaurus Translation

**Neuron-Glial  
Markers**  
Astrocytes, GIIa,  
Oligodendrocytes,  
Neuron and  
Schwann Cells  
Markers  
[www.neuromics.com](http://www.neuromics.com)  
Ads by Google

☒ English
 ☐ All Languages
 ☐ Choose Language

Synonyms +	Translations	Timelines	Bibliography	Quotations +
Geography	Slats	Anagrams	Rhyming	Images & SFX
Eve	Games	Poetry	News	www Search

Earth's largest dictionary with more than 1226 modern languages and Eve!

Memory  
Attention

Focus  
Speed

**Spatial Reasoning**  
**Problem Solving**






## Play Games

 **Catalan:** neurites.

Part of Speech	Definition
Noun	1. An axon. <a href="#">[Eve - graph theoretic]</a>

Sources: compiled from various sources, (under license) copyright 2008.

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**"Neurite" is a common misspelling or typo for: nitrite, neuritis, neurites.**

Any projection from the cell body of a neuron can be referred to as a **neurite**. This projection can be either an axon or a dendrite. The term is frequently used when speaking of immature or developing neurons, especially of cells in culture, because it can be difficult to tell axons from dendrites in that situation.

Neurites are often packed with microtubule bundles, the growth of which is also mediated by the latter.



Source: adapted by the editor from Wikipedia, the free encyclopedia; from the article "Neurite". Image Credit

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Topics sorted by level of Interest	Level (1=low, 600=high)	Topics sorted Alphabetically	Level (1=low, 600=high)
Neurite	2	Neurite	2

Source: the editor, created by/for EVE to gauge likely levels of human interest in linguistically triggered topics (compiled across various sources, such as Wikipedia and specialty expression glosses).

Position	Synonym (sorted by strength)
Noun	axon.

Source: Eve, based on meta analysis.

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Rank	Intensity	Word	Synonyms	Synonyms of synonym
1	1.0092	neurite	axon	neuron, axone, Deiters process, Exon, axis cylinder

Source: calculated by Eve using graph theory. "Intensity" is a score indicating the number of overlapping cliques where the word pair is found (an integer before the decimal); the first digit after the decimal is the number of overlapping terminal characters up to 9; the second characters is number of leading common characters up to 9; the last two digits measure the Levenshtein distance subtracted from 100.

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Language	Translations (or nearest inflections or synonyms, in parentheses)
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Chinese Simplified 轴突 (axon, axone, neurite). Additional references: [Chinese Simplified](#), [China](#), [Brunei](#), [neurite](#). (volunteer & more translations)

Deutsch **Neurit** (neurite). Additional references: Deutsch, Germany, Austria, neurite. (volunteer & more translations)

German      **Neurit** (neurite). Additional references: German, Germany, Austria, neurite. (volunteer & more translations)

High German      **Neurit** (neurite). Additional references: High German, Germany, Austria, neurite. (volunteer & more

	<a href="#">translations</a> )
Hochdeutsch	<b>Neurit</b> (neurite). Additional references: <a href="#">Hochdeutsch</a> , <a href="#">Germany</a> , <a href="#">Austria</a> , <a href="#">neurite</a> . ( <a href="#">volunteer &amp; more translations</a> )
Japanese	<b>神経突起</b> (neurite). Additional references: <a href="#">Japanese</a> , <a href="#">Japan</a> , <a href="#">Taiwan</a> , <a href="#">neurite</a> . ( <a href="#">volunteer &amp; more translations</a> )

Source: [Evg.](#) based on a combination of meta analysis and graph theory (for near and back translations).

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### Constructed Language Translations: neurite

Language	Translations for "neurite" or closest synonym(s); back translations in parentheses.
Athag	<b>nathageurathagite</b> (neurite). Additional references: <a href="#">Athag</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )
Double Dutch	<b>nageuragite</b> (neurite). Additional references: <a href="#">Double Dutch</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )
Leet	<b>£_ z1- -£</b> (neurite). Additional references: <a href="#">Leet</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )
Oppish	<b>nopeuropite</b> (neurite). Additional references: <a href="#">Oppish</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )
Pig Latin	<b>euritenay</b> (neurite). Additional references: <a href="#">Pig Latin</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )
Terran B	<b>Neurit</b> (neurite). Additional references: <a href="#">Terran B</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )
Ubbi Dubbi	<b>nubeurubite</b> (neurite). Additional references: <a href="#">Ubbi Dubbi</a> , <a href="#">neurite</a> . ( <a href="#">volunteer</a> )

Source: compiled by [the editor](#).

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### Volunteer Translation: English(🇬🇧) to French(🇫🇷)

#### Moderated translations:

English(🇬🇧)	French(🇫🇷)	Ratings
neurite	neurites	1 <a href="#">f</a> <a href="#">t</a> 0
neurite	neuritique	1 <a href="#">f</a> <a href="#">t</a> 0
neurite	Neurite	1 <a href="#">f</a> <a href="#">t</a> 0

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### Volunteer Translation: French(🇫🇷) to English(🇬🇧)

#### Moderated translations:

French(🇫🇷)	English(🇬🇧)	Ratings
neurites	neurite	1 <a href="#">f</a> <a href="#">t</a> 0

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### Adjacent words:

<a href="#">Neurasthenic</a>	<a href="#">Neurilemoma</a>	<a href="#">Neuroanatomic</a>
<a href="#">Neurasthenically</a>	<a href="#">Neurility</a>	<a href="#">Neuroanatomical</a>
<a href="#">Neurasthenicly</a>	<a href="#">Neurine</a>	<a href="#">Neuroanatomicly</a>
<a href="#">Neurath</a>	<a href="#">Neurinoma</a>	<a href="#">Neuroanatomy</a>
<a href="#">Neuration</a>	<a href="#">Neurism</a>	<a href="#">Neurobiological</a>
<a href="#">Neurectomy</a>	<a href="#">Neurite</a>	<a href="#">Neurobiologically</a>
<a href="#">Neurenteric</a>	<a href="#">Neuritic</a>	<a href="#">Neurobiologies</a>
<a href="#">Neurenterically</a>	<a href="#">Neuritis</a>	<a href="#">Neurobiologist</a>
<a href="#">Neurentericly</a>	<a href="#">Neuro</a>	<a href="#">Neurobiology</a>
<a href="#">Neuridin</a>	<a href="#">Neuro-</a>	<a href="#">Neuroblast</a>
<a href="#">Neurilemma</a>	<a href="#">Neuroactive</a>	<a href="#">Neuroblastoma</a>

### Web Search Results: neurite

Google

Web [www.websters-online-dictionary.org](#)